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REVIEW ARTICLE

Oncogenes and anti-oncogenes in human epithelial thyroid tumors

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INTRODUCTION

Thyroid tumors represent an appropriate model for the study of the epithelial neoplastic transformation. Indeed, different types of tumors (benign adenomas, differentiated and anaplastic carcinomas) have been individualized, *in vitro* models which reproduce different degrees of neoplastic transformation are available and functional parameters (i.e. iodine uptake, hormone production, response to TSH stimulation) can be studied *in vivo* or *in vitro*. A role of somatic mutations, gene rearrangement(s) and level of gene expression in carcinogenesis is now well established. Several techniques can be used to detect such genetic alterations in human tumors: 1) the gene transfer assay, which detects dominant oncogenes capable of inducing malignant transformation of receptor rodent cells (e.g. *ras, ret, trk*); 2) the polymerase chain reaction (PCR) amplification, followed by differential oligonucleotide probing and/ or direct sequencing that detect mutations; 3) hybridization with specific probes, able to detect polymorphisms or rearrangements in cellular DNA digested with specific restriction enzymes and 4) the Northern, immunoprecipitation and Western techniques, able to determine the level of expression of a gene.The conjunction of these techniques applied to thyroid tumors, has focused particular attention on the role of mutations activating the oncogenes *ras* and *gsp* (1-6), rearrangements activating the oncogenes *ret* and *trk* (7-10) and alterations in the pattern of expression activating the oncogene *met* (11). In this review we will analyze results concerning the frequency of activation of *ras, gsp, ret, trk* and *met* and discuss whether these oncogenes play an alternative or a complementary role in thyroid tumorigenesis. We will also discuss the role of tumor-suppressor genes in this process.

RAS ONCOGENES

The activation of the *ras* oncogenes by point mutation, was found in about 40% of the thyroid tumors and was the most frequent genetic alteration (Table 1). Therefore, thyroid tumors are together with pancreas, colon and Xeroderma pigmentosum skin tumors, among those presenting a high frequency of *ras* activation (12-14). The mutations are randomly distributed between the 3 *ras* genes with similar frequencies (11-15%), without predominance of one of the critical codons or their constituting bases. Mutations occur in follicular adenomas, papillary and follicular carcinomas and anaplastic carcinomas, at approximately the same frequency. In contrast to another study (2), we have found a high frequency of *ras* mutations in macrofollicular adenomas (4) in accordance with a previous finding of such mutations in multinodular goiters (15). Indeed, the thyroid gland is, along with the colon, a tissue in which *ras* mutations are observed in benign tumors (2, 4, 13).

Although derived from the same cell type as its follicular counterpart, papillary carcinomas show a different biological behaviour (16). Comparatively to other studies, a higher frequency of *ras* mutations was found by us in this type of tumor (Table 1). As previously stated (4), this discrepancy is not due to differences in sensitivity of the analytical techniques used (PCR followed by hybridization with synthetic probes), because the overall frequencies of *ras* mutations detected are not widely divergent among the different series. It may be the consequence of a difference in iodine intake. Indeed, in our series the frequency of *ras* mutations in papillary tumors is lower in patients living in io-

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Table 1 - *Ras mutations in human thyroid tumors.*

	PC*	FC	AC	AD(cold)	HN
Canada (52)	0** (0/10)	10 (1/10)	–	20 (2/10)	–
Cardiff (43)	21 (4/19)	52 (11/21)	55 (6/11)	27 (8/29)	–
Hungary (52)	0 (0/12)	50 (3/6)	–	92 (12/13)	–
Milan (8)	13 (2/16)	–	–	–	–
Naples (7)	0 (0/20)	–	–	–	–
USA (15)	21 (3/14)	0 (0/3)	–	25 (6/24)	–
USA (53)	6 (1/15)	14 (2/14)	–	0 (0/9)	–
Our study	45 (16/35)	35 (7/20)	100 (1/1)	40 (12/30)	7 (2/28)
Overall	18 (26/141)	32 (24/74)	58 (7/12)	35 (40/115)	7 (2/28)

*: abbreviations: PC: papillary carcinoma; FC: follicular carcinoma; AC: anaplastic carcinoma; AD: cold adenoma; HN: hot nodule

**: %frequency of activation. In parentheses: positive tumors/studied tumors.

dine deficient areas (25%: 3/12), than in those living in areas with apparently sufficient iodine intake (56%: 13/23).

In consequence, *ras* which as stated can be found mutated in either adenomas or carcinomas at roughly the same frequency, can be proposed as an early event of the thyroid tumorigenic process. However, tumor DNA analysis alone cannot prove an initiating role of *ras* mutation. Support for this hypothesis has therefore been obtained from experiments in which mutant *ras* has been introduced into normal follicular cells either *in vitro* or *in vivo*. *In vitro*, introduction of mutant *ras* into primary cultures of either human or rat follicular cells (16), results in the stimulation of proliferation, with increase of the number of cells in S phase, extension of the proliferative lifespan and no detectable loss of tissue-specific markers (mimicking a follicular adenoma). *In vivo*, in transgenic mice, thyroid hyperplasia and papillary carcinomas were obtained in our laboratory using a vector in which the bovine thyroglobulin (Tg) promoter drives the expression of a mutated Ha-*ras* oncogene (Rochefort et al., submitted). Moreover, thyroid adenomas and a follicular carcinoma, were obtained using a vector in which the rat Tg promoter drives the expression of a Ki-ras activated gene c-DNA (17). Altogether these data are consistent with a role of *ras* as an early event in any histological type of thyroid tumor, but also suggest that other still unknown genetic alterations participate in the determination of the histological type of the tumor (Fig. 1). The activated *ras* protein stimulates the growth and inhibits differentiation of thyroid epithelial cells (2, 4, 16).

GSP ONCOGENE

The overall frequency of *gsp* mutations in codons 201 (exon 8) or 227 (exon 9) is 10%: about 30% in hot nodules, and 8% in non functioning tumors (7% in adenomas and 9% in differentiated carcinomas) (Table 2). The frequency of mutations in hot nodules was lower in the studies from Lyons et al. (5). *Gsp* activation through cAMP overproduction, stimulates both cell growth and cell differentiation (16), correlating with its relatively high frequency of mutation in hot nodules (18; our study However, about 70% of hot nodules are negative for a *gsp* mutation. It was therefore necessary to determine whether in hot nodules negative for mutations in exons 8 and 9, *gsp* can be activated by mutations in other exons or replaced by genetic alterations in genes not yet considered as an oncogene and participating in the cAMP pathway, such as the genes

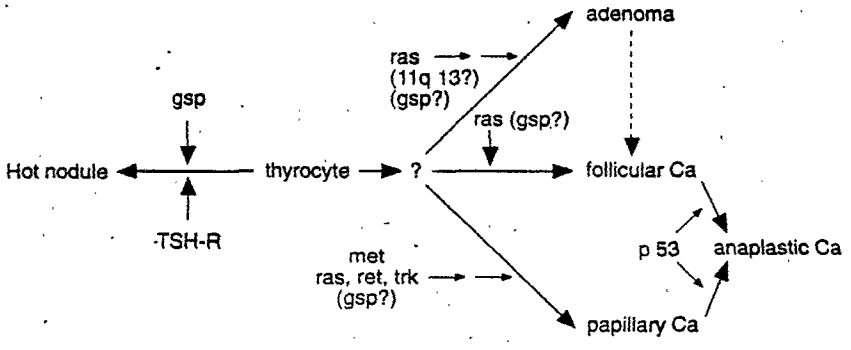

Fig. 1 - *Somatic genetic events in multistage epithelial thyroid tumorigenesis.*

Table 2 - *GSP oncogene in human thyroid tumors.*

	AD (cold)*	HN	PC	FC	AC
USA (5)	0** (0/12)	7 (1/14)	0 (0/4)	0 (0/7)	0 (0/1)
London (18)	0 (0/16)	38 (5/13)	0 (0/5)	0 (0/3)	–
Japan (19)	0 (0/24)		13 (4/30)		0 (0/2)
Our study	7 (2/30)	32 (9/28)	9 (3/35)	10 (2/20)	0 (0/1)
Overall	2 (2/82)	27 (15/55)	11 (7/74)	7 (2/30)	0 (0/4)

*: abbreviations are as in Table 1

**: percent of point mutations. In parentheses: positive tumors/studied tumors.

for TSH receptor (TSH-R), adenylate cyclase or protein kinase A. Indeed, mutations in codons 619 and 623 in the loop III of the TSH-R gene have been recently found in thyroid hot nodules by Parma et al. (19) and only in codon 623 in our laboratory (Russo et al., 1994, in press).

As stated in Table 2, *gsp* mutations were also found in hypofunctioning tumors (20 and our data). In a previous report (6), we communicated the detection of *gsp* mutations in 3/6 carcinomas with elevated basal cAMP and no further increase following TSH stimulation. Twenty three other carcinomas with low or normal basal cAMP levels and which could be stimulated by TSH, were negative. These data together with those presented in Table 2, suggest that *gsp* mutations may also participate in the tumorigenesis of hypofunctioning thyroid tumors. In fact, a sustained elevation of cAMP leads *in vivo* to transitory growth of normal follicular cells (16). In non-functioning tumors, the *gsp* mutation may confer a growth advantage to a cellular clone in which another yet unknown genetic lesion had already abrogated a growth-limiting mechanism, which normally downregulates the response to cAMP (Fig. 2). This could be in accordance with our finding of a simultaneous presence of *ras* and *gsp* mutations in a papillary carcinoma (see below). Conversely, the presence of *ras* mutations in 2/12 hot nodules may be related to an associated occult tumor or the presence of two cell populations in a single tumor. It may also be consistent with another genetic abnormality that masks the inhibition of cell function induced by *ras* mutation.

In conclusion, *gsp* can now be considered as an initiator for a minority of "hot nodules" (about 30%), but its role in the developpement of other thyroid tumors is much less certain (Fig. 1).

ONCOGENES FROM THE TYROSINE PROTEIN KINASE RECEPTORS FAMILY

Since the discovery of tyrosine kinases over a decade ago, this gene superfamily has been steadily growing to reach its current size of almost 50 members (21). A significant fraction of these genes code for cell surface glycoproteins that function as growth factor receptors. They include the receptors for insulin, IGF-1, EGF, HGF-SF, PDGF A and B, M-CSF, the product of the "steel" gene, and the members of the FGF family. Structurally related tyrosine kinase genes such as *eck, eph, ret, ros,*

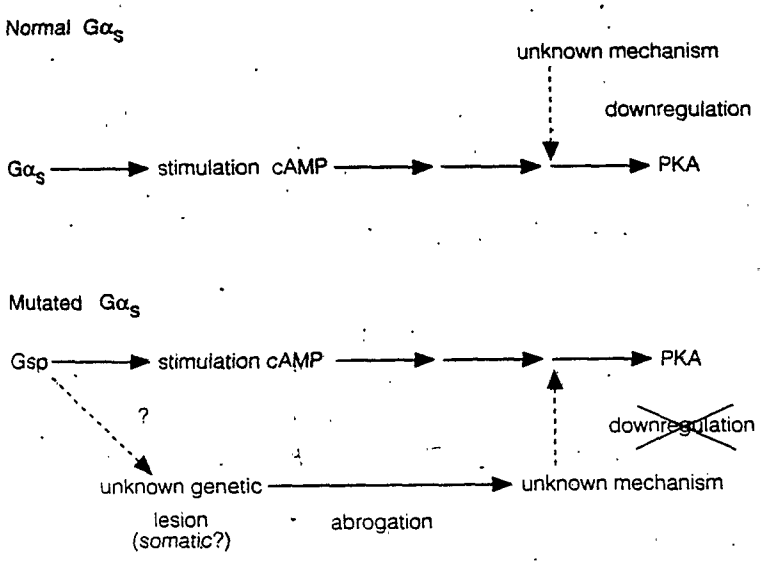

Fig. 2 - *Presence of gsp mutations in thyroid hypofunctioning tumors.*

sea, etc., are also thought to code for cell surface receptors, although their putative cognate ligands remain to be identified (21). The members of the *trk* family (*trk* C and *trk* D) and *c-met* belong to the latter group of tyrosine kinases (21).

A- RET ONCOGENE

The activation of the *ret* oncogene in thyroid tumors was first demonstrated by Fusco et al. (7). The activated oncogene was present only in papillary carcinomas with a frequency of more than 20% and was called *PTC* (10 and Table 3). This gene derives from the fusion of the tyrosine kinase domain of the *ret* proto-oncogene and the 5' terminal region of another gene named H4. A coding sequence 354 bp long that belongs to the H4 (D10S170) gene replaces the truncated transmembrane and extracellular domains of the *ret* proto-oncogene. An intrachromosomal rearrangement generates the chimeric *ret/ PTC* oncogene. In fact both H4 and *ret* genes are located on the long arm of chromosome 10, 10q 11.2 and q21 respectively, at a distance of at least 280 kb. A chromosomal inversion (q11.2-q21) is responsible for their fusion (9, 10, 22). More than 100 thyroid tumors other than papillary carcinomas studied, were negative for *ret* activation (22). The same result was obtained analysing 600 non-thyroid neoplasias. Recently, other isoforms of the ret/PTC oncogene have been isolated from human thyroid papillary carcinomas: the *PTC 2* and *PTC 3* genes. In the *PTC 2* oncogene, the 5' part of the gene was represented by the regulatory subunit RI of the protein kinase A gene (23); in the *PTC 3* the tyrosine kinase domain of the gene fused with an unknown gene named rfg (A. Fusco, personal communication).

Table 3 - *Ret oncogene in human thyroid tumors.*

	AD (cold)*	HN	PC	FC	AC
Cardiff (16)	0** (0/26)	–	7 (2/30)	0 (0/9)	0 (0/4)
Naples (10)	0 (0/16)		33 (14/42)	0 (0/13)	0 (0/8)
Japan (23)	21 (4/19)	–	9 (1/11)	–	–
Lyon (10)	0 (0/18)	–	11 (8/70)	0 (0/13)	0 (0/5)
USA (10)	–	–	17 (11/65)	0 (0/11)	0 (0/2)
Our study	0 (0/30)	0 (0/12)	10 (2/19)	0 (0/6)	0 (0/1)
Overall	4 (4/109)	0 (0/12)	16 (38/237)	0 (0/52)	0 (0/20)

*: abbreviations are as in table 1.

**: percent of rearrangements. In parentheses: positive tumors/studied tumors.

The overall frequency of *ret* activation is 16%, with variations among the different studies. The higher frequency was observed in the Italian series (Table 3). All the rearranged *ret* genes, were detected in papillary carcinomas with the exception of a report by Ishizaka et al. (24). Indeed, these authors reported the detection of an activated *ret* oncogene in 4 out of 16 adenomas. The prevalence of occult thyroid carcinomas in the Japanese population being high, it is possible that the *ret*-positive tumor samples are the consequence of the presence of an occult papillary tumor. This hypothesis is strengthened by the fact that the activation of *ret* was detected only in some regions of the tumors.

B- TRK ONCOGENE

The human *trk* gene encodes a cell surface tyrosine kinase (tk) protein representing one of the receptors for the nerve growth factor (NGF) (21, 25). The expression of *trk* is tightly controlled, being restricted to the peripheral nervous ganglia (26). *Trk* undergoes oncogenic activation through the formation of a chimeric fusion protein. Following chromosomal rearrangement(s), the *trk* 5' region is removed and replaced by sequences provided by an activating gene (21), whose expression is ubiquitous. The resulting chimeric gene is also ubiquitously expressed and its tk domain constitutively active, these features representing, presumably, the bases for its transforming activity. The human *trk* gene was first identified as an oncogene, in a human colon carcinoma (27). The malignant activation of the gene, was the consequence of a somatic rearrangement in chromosome 1, that fused 7 exons of the non-muscular tropomyosin gene, with the tk domain of *trk* (21). However, other types of *in vitro* or *in vivo* activating rearrangements, have also been described (21).

The *trk* oncogene has been found activated until now only in papillary carcinomas (8, 28 and IXth Meeting on Oncogenes, Frederick, Md., USA, 1993). In our laboratory, only 2 *trk* activating rearrangements were found among the 68 thyroid tumors studied by digestion with restriction enzymes and hybridization with a specific *trk* probe: one in a papillary carcinoma and one in a lymph node metastasis of an anaplastic carcinoma derived from a papillary carcinoma (Table 4). The frequency of *trk* activation in the overall series as well as that calculated when only the papillary carcinomas are considered, is lower than in the Italian study. Further studies are neces-

Table 4 - *TRK oncogene in human thyroid tumors.*

	AD (cold)*	HN	PC	FC	AC
Italy**	–	–	15° (8/52)	–	–
Our study	0 (0/30)	0 (0/12)	5 (1/19)	0 (0/6)	100# (1/1)

*: abreviations are as in Table 1

**: Proc. of the IX th. Meeting on Oncogenes, Fredericks; Md., USA. 1993

°: percent of rearrangements. In parentheses: positive tumors/studied tumors.

#. lymphoid metastasis of an anaplastic carcinoma derived from a PC.

sary to determine whether this difference is also a consequence of geographic factors. In our two positive tumors, the *trk* activating rearrangement found by Bam HI DNA digestion, corresponded to that described by Barbacid et al. (21), as a rearrangement between the 5' region of the non-muscular tropomyosine gene and the *trk* proto-oncogene 3' region. The possibility cannot be excluded that using the transfection technique we may have found more *trk* activating rearrangements, including those recently described in papillary thyroid tumors by Greco et al. (28) between *tpr* and *trk* resulting in the *trk-T1* and *trk-T2* oncogenes. As cited above, the *trk* rearrangement in one of our positive tumors was found in a metastasis of an anaplastic carcinoma, together with a *ras* mutation. In the primary tumor only the ras mutation was present. The non-muscular tropomyosine-*trk* rearrangement could be the consequence of an internal rearrangement in chromosome 1, produced during the metastatic process.

C- MET ONCOGENE

The c-*met* oncogene encodes a transmembrane tyrosine kinase protein identified as the receptor for a polypeptide known as Hepatocyte Growth Factor (HGF) or Scatter Factor (SF) (33, 34). HGF-SF is a potent mitogen for epithelial cells and promotes cell motility and invasion (35). The *met*/HGF-SF receptor is a 190 kDa heterodimer with two disulphide-linked subunits: an extracellular 50 kDa α chain and a transmembrane 145 kDa ß chain, showing tyrosine kinase activity (36, 37). The receptor is synthesized as a 170 kDa precursor that is glycosylated and cleaved to give the mature heterodimer (37). The oncogenic potential of *met* may be activated by truncation and fusion with unrelated sequences which mediate aberrant dimerization (38, 39) and by amplification and overexpression (11) or defective post-translational processing (40).
In epithelial thyroid tumors, the expression of the met-HGF receptor was investigated at the protein level, by P. Comoglio et al. (Torino, Italy), using the Western blot analysis (11 and International Workshop on Thyroid Cancer: Basic Science and Clinical Problems, Taormina, Italy, 1993). Fifty three carcinomas (30 papillary, 14 follicular, 4 poorly differentiated and 5 anaplastic) and 21 adenomas were studied. The *met* oncogene was overexpressed in 50% of the thyroid carcinomas, mainly in the papillary tumors (21/30: 70%). In the other histological types of tumors, the frequency of overexpression was null or low. It has been proposed that overexpression of the met-HGF receptor by neoplastic follicular cells, might sustain their growth through the action of the ligand. Moreover, it has also been postulated that this stimulation of the thyroid follicular cell growth, would be a paracrine one, since it has been shown by Zarnegar et al. (41) that HGF-SF is secreted by the thyroid parafollicular-C cells.
In conclusion, concerning the alteration of oncogenes of the tyrosine protein kinase receptors family, in thyroid tumors:
1- the results show that *ret* and *trk* rearrangements are apparently restricted to papillary carcinomas, playing the role of an initiator in a minority of these tumors (Fig. 1). The major question is why are these genes involved in follicular tumors or medullary tumors at all. *Ret* and *trk* expression is limited essentially to neuroectodermal cell types (26, 29, 30). The expected target cell should therefore be the thyroid C cell and not the follicular cell. One explanation could be that as already proposed (31), some thyrocytes share two alternative differentiation states: neuroendocrine or glandular. If this is the case, some epithelial cell tumors might express part of the growth signal pathways of the normal C cell, as happens in some "endocrine" tumors of the lung (32). This would justify the paradox that *ret* and *trk* have not been found rearranged in medullary thyroid tumors, but cannot explain why they are restricted to papillary epithelial tumors;
2- the data also suggest a role for the overexpression of c-*met* in the pathogenesis and progression towards malignancy, through the acquisition of a more aggressive behaviour, of thyroid epithelial tumors mainly of the papillary histotype (Fig. 1). Whether in some of these tumors the "scatter" action of HGF plays a role in the formation of metastases has yet to be confirmed.

ONCOGENES IN THYROID RADIATION-ASSOCIATED TUMORS

The study in our laboratory in 11 radiation-associated tumors of the *ras*, *gsp*, *ret* or *trk* oncogenes,

Table 5 - *Ras mutations in human radiation-associated thyroid tumors.*

	Cardiff (43)	Our study	Total
Follicular adenoma	0/4*	1 N-ras/5	1/9
Follicular carcinoma	3 Ki-ras/5	0/2	3/7
Papillary carcinoma	1 N-ras/2	1 N-ras/4	2/6
Anaplastic carcinoma	0/1	–	0/1

*: positive tumors/studied tumors. In parentheses: overall frequency.

showed 2 *ras* mutations and 2 *gsp* mutations. Both *ras* positive tumors presented a mutated N-*ras* gene, in codon 12 and in codon 61 respectively. This result is in contradiction with previous data (42, 43) according to which, in both rat and human thyroid, the Ki-*ras* oncogene was preferentially activated by radiation. When all available data are pooled (Table 5), it appears that in radiation-associated human thyroid tumors, two oncogenes (*ras* and *gsp*) are activated by point mutation of one of the critical codons, with about the same frequency as in "spontaneous' tumors, and that Ki- and N-*ras* are the most frequently activated oncogenes, apparently with similar frequencies.
However, to confirm these preliminary results and to also have a more precise idea concerning the eventual activation of *ret* and *trk* oncogenes, the analysis must be extended to larger series of human radiation-associated thyroid tumors.

COMBINED STUDY OF THE RAS, GSP, RET AND TRK ONCOGENES

Genetic alterations in the same sample of the *ras*, *gsp*, *ret* and *trk* oncogenes were sought by us in 68 benign and malignant thyroid tumors (Table 6). Two simultaneously altered oncogenes were detected in only 2 tumors: one was a papillary carcinoma bearing a N-*ras* and a *gsp* mutation and the other was the lymph node metastasis from an anaplastic carcinoma showing a Ki-*ras* mutation together with a *trk* rearrangement, absent in the primary tumor (see above). This result suggests that the studied oncogenes can play an alternative role in thyroid tumorigenesis (Fig. 1) and that there is no significant association between *ras* and *gsp* or *trk* genetic alterations.
These data nevertheless show that the presence of more than one genetic abnormality affecting the four studied oncogenes at a given stage of thyroid tumorigenesis is possible.

Table 6 - *Study of four oncogenes in 68 human thyroid tumors.*

- One or more altered oncogenes were found in 37/68 tumors:
  - Carcinomas: 17/26 (65%)*
  - Adenomas: 14/30 (47%)*
  - Hot nodules: 6/12 (50%)*
- In only two tumors, two simultaneously altered oncogenes were found:
  - 1 Papillary carcinoma: N-ras+gsp
  - 1 Lymph node metastasis from an anaplastic carcinoma: Ki-ras+trk

*: Positive tumors/studied tumors. In parentheses overall frequency.

TUMOR SUPPRESSOR GENES

While oncogene alterations are usually dominant (i.e. ras single point mutations leading to uncontrolled cellular growth), tumor suppressor genes become tumorigenic through loss of function and tend to act in a recessive manner. The best example of this type of genes is provided by the retinoblastoma gene *Rb* (44). Alteration(s) in tumor suppressor genes are frequently related to mutations and/or deletions in tumoral chromosomal material. However, until now, these type of events seems to be relatively infrequent in human thyroid tumors.
p^{53} is by far the gene most often modified in human cancer, being found altered at high frequency in tumors of colon, breast, lung, in acute leukemia, etc (45). The mutations in this gene, as opposed to *ras* or *gsp* mutations, can occur at multiple sites in the evolutionary conserved regions of the molecule (45). In thyroid tissues, inactivating point mutations in the p^{53} gene were observed with a high frequency in anaplastic but not differentiated carcinomas (46, 47). These data suggest that mutational inactivation of the p^{53} gene may be a key event in the progression from differentiated to anaplastic carcinoma (Fig. 1). On the contrary, no genomic abnormalities were found by Southern blot in the *Rb* gene, in different series of thyroid tumors studied (ref.16 and 40 benign and malignant tumors studied in our laboratory). Clinical studies have shown an increased incidence of thyroid tumors in patients with colon polyposis and Gardner's syndrome, and in those with Cowden's disease or multiple endocrine neoplasia (MEN) type 1. Whether this association of thyroid tumor(s) with colon polyposis is directly related to an alteration of the APC gene (48) remains to be studied. No mutation in the APC gene has been so far described in sporadic differentiated tumors of the thyroid gland. Loss of genetic sequences in the long arm of chromosome 11 (11q13) (49), has been described in some sporadic follicular but not papillary thyroid

Fig. 3 - *Somatic events in multistage epithelial colon tumorigenesis.*

neoplasms. This region is related to several genes associated with tumorigenesis, notably, the gene conferring a predisposition to multiple neoplasia type 1 (MEN 1). It has been suggested (49) that the loss of a gene located in the 11q13 region (the MEN 1 gene?) may direct progression to a follicular phenotype, the genetic alteration being, perhaps, common to benign and malignant follicular tumors (Fig. 1). Finally, a loss of genetic material on chromosome 3, has also been described, but only in some follicular carcinomas (50).

CONCLUSIONS AND PERSPECTIVES

From the results discussed in this review, it appears that a very similar model can be proposed to explain the initiation and progression of the thyroid and colon tumorigenic process. However, a difference exists in the genes involved in the phenotypic progression (51). Indeed, in colon adenomas, the *ras* mutations seem to appear later than in thyroid adenomas, and it has been suggested (48, 51) that the true initiators are genes belonging to a new family: APC/ MCC (Fig. 3).

Moreover, the fact that one of four genes *ras, gsp, ret,* or *trk* is activated in a thyroid tumor suggests: 1) an interchangeable role for these genes in tumor initiation or progression (Fig. 1) and 2) that the simultaneous activation of 2 genes is a rare event, but may lead to a super-added effect of the combination. However, the requirement of one of the 4 oncogenes to interact with other genes involved in thyrocyte growth (i.e. IGF-1) (16), or the participation of other genes in the initiation or progression of the thyroid carcinogenetic process (i.e. MEN 1 genes?), must not be neglected (Fig. 1). In this respect, the frequent occurrence of p53 gene mutations in poorly differentiated and anaplastic tumors is worth noting (46, 47).

Further identification of new oncogenes or tumor suppressor genes as well as a better knowledge of the physiology of the normal follicular cell in terms of cell proliferation, differentiation and expression of differentiated functions, are needed to open new avenues in the biology and clinical management of thyroid tumors.

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